

## Definitions of Pathologic Features and Grading Criteria

Each slide of upper lobe lung parenchyma (blocks D, E, F, G and H) will be categorized separately. A single section from the lower lobe will be analyzed (block K).

Separate grades for each section or slide will be given for each of the following features.

### **Macules**

Collections of dust-laden macrophages in a size range of 1.0 to 6 mm within the walls of respiratory bronchioles and adjacent alveoli enmeshed within a fine network of reticulin and occasional collagen fibers, associated with focal emphysema.

- 0 No lesions.
- 1 Peribronchial dust deposition without significant fibrosis.
- 2 Macules as defined above and involving less than 33% of respiratory bronchioles.
- 3 Macules involving 33-66% of respiratory bronchioles.
- 4 Macules involving 67% or more of respiratory bronchioles.

**NOTE:** There is some overlap between macules and small airways disease involving the respiratory bronchioles. Treat both as independent entities.

### **Focal Emphysema**

Focal emphysema involves the centriacinar portions of the lobule.

- N No focal emphysema seen in section.
- Y One or more macules show how focal emphysema.

**NOTE:** Focal emphysema is considered to occur only around macules. Do not include other types of emphysema; e.g., panacinar, scar, etc.

## **Nodules**

Lesions up to 10 mm (1 cm) in size with round, irregular or serpiginous borders with a fibrotic stroma of collagen and reticulin containing dust-laden macrophages. Nodules in subpleural and peribronchial locations are included.

- 0 Nodules absent.
- 1 Single or occasional nodules occupying less than 10% of parenchyma.
- 2 Nodules occupying from 10 to less than 33% of parenchyma.
- 3 Nodules occupying 33-66% of parenchyma.
- 4 Nodules occupying 67% or more of parenchyma.

## **Nodule Types**

### **Silicotic (S)**

Nodular lesions with smooth borders and a laminated appearance, or a conglomeration of such lesions within the size constraint. A silicotic nodule *must have* concentrically arranged collagen around its center.

### **Mixed Dust (M)**

Nodular lesions with irregular borders and interlacing bundles of collagen enclosing pigmented macrophages.

### **Granulomatous (G)**

Nodular lesions with smooth or irregular borders. The collagen has an irregular arrangement and there is a marked giant cell and histocytic response to the dust. The latter should contain brightly birefringent silicate particles to distinguish from sarcoid and infectious granulomata.

## **Interstitial Fibrosis**

Diffuse or irregular fibrosis of alveolar septa and/or alveolar ducts.

- 0 No alveolar wall thickening.
- 1 Minimal fibrosis.
- 2 Category 1 plus coalescence of fibrotic change between two adjacent acini (diffuse interstitial fibrosis).
- 3 Category 2 plus architectural restructuring and/or cyst formation (honeycombing).

### **Emphysema**

- 0 No emphysema.
- 1 Increased focal points of destruction in alveolar walls or ducts (fenestrae) without alterations in alveolar architecture.
- 2 As 1, with two or more isolated islands of tissue in the centriacinar zone.
- 3 As 2, plus distortion and/or enlargement of alveolar or ductal spaces.

**NOTE:** As the severity of emphysema in this age group is expected to be early/mild, the criteria of M. Saetta *et al.* (*Am Rev Res Dis* 131:764-769, 1985) will be used. For grade 1, “increased” is defined as two or more fenestrae in any alveolus or alveolar duct *and* a majority of acini affected. Normal fenestrae or pores of Kohn are very rarely encountered in a single transverse section of an airspace. Therefore, two or more is considered abnormal.

### **Lymph Node Changes**

- 0 No fibrotic changes.
- 1 Occasional small areas of medullary fibrosis.
- 2 Occasional discrete hyalinized nodules in cortex or medulla.
- 3 Confluent nodules occupying more than 50% of lymph node.

### **Pleural Thickening**

- 0 No thickening.
- 1 Patchy pleural thickening.
- 2 Diffuse pleural thickening not exceeding 0.5 mm in thickness.
- 3 Diffuse pleural thickening exceeding 0.5 mm in thickness.
- 4 Any of the above together with pleural nodules.

### **Small Airways**

*Membranous bronchioles:* airways < 2 mm internal diameter, without cartilage in walls, non-alveolated.

*Respiratory bronchioles:* small airways with alveolated walls, extending in 3 generations from terminal membranous bronchiole to alveolar ducts.

Airways were compared to standard photomicrographs of grades.

### Grading of Small Airway Disease

All small airways in a section fulfilling the definitions above are classified as membranous or respiratory and graded for the following:

- a) fibrosis
- b) muscle hypertrophy
- c) wall inflammation
- d) pigment
- e) goblet cell metaplasia
- f) the respiratory bronchioles are also evaluated for intra-luminal macrophages.

Orientation of the airway is not considered. Airways that extend off the edge of the slide are not graded (based on the grading system of Wright *et al.*, *Arch Pathol Lab Med* 109:163-165, 1985). Each generation of respiratory bronchioles is identified as RB1, RB2 and RB3. Bronchioles that cannot be so identified are not graded.

#### a) Fibrosis

This is graded primarily on the elastic trichrome stain.

- Grade 0: Normal, thin wall.
- Grade 1: Expansion of the wall by loose connective tissue.
- Grade 2: Further expansion with condensation of collagen.
- Grade 3: Dense connective tissue with or without *thinning* of wall.

#### b) Muscle Hypertrophy

- Grade 0: Normal.
- Grade 1: Mild increase in smooth muscle.
- Grade 2: Moderate increase in smooth muscle.
- Grade 3: Severe increase in smooth muscle.

#### c) Inflammation

Grading is based on number of chronic inflammatory cells (lymphocytes, plasma cells, macrophages, eosinophils) in wall. Ignore acute inflammatory cells.

- Grade 0: No inflammation.
- Grade 1: Mild.
- Grade 2: Moderate
- Grade 3: Severe (includes lymphoid follicles).

d) Pigment

Opaque and birefringent particles within the airway walls are graded separately.

Opaque particles: 0 = none  
1 = small quantities  
2 = moderate amounts  
3 = dense deposits

Birefringent particles: 0 = none  
1 = small quantities  
2 = moderate amounts  
3 = dense deposits

e) Goblet Cell Metaplasia (membranous bronchioles only)

0 = none  
1 = mild increase  
2 = moderate increase  
3 = severe increase

f) Intraluminal Macrophages (respiratory bronchioles only)

Macrophages within lumen of respiratory bronchioles or adjacent alveoli are included.

0 = none  
1 = occasional  
2 = moderate number  
3 = large number

## **Lung Mineral Dust Analysis**

### **1) Bulk analysis: X-Ray diffraction analysis for crystalline silica**

At UC Davis, 5 g samples from the fixed lung were systematically collected as described in the methods section of the manuscript and sent to Dr Val Vallyathan, Ph.D. at NIOSH, Morgantown, WV. The samples were extracted using combinations of bleach digestion, lipid extraction and low temperature plasma ashing (Pooley, 1981). A sample blank for each lung tissue specimen was carried through each step of the preparation. The bulk dust samples were analyzed by x-ray diffraction using the powder x-ray diffraction technique as described for airborne silica samples according to NIOSH method NMAM-7500 (Fourth Edition, 8/94) by DataChem Laboratories, Salt lake City, UT. Samples and standards were run concurrently and an external calibration curve prepared from the integrated intensities. Total dust, total mineral dust and total silica dust concentrations were expressed as grams and/or micrograms per gm dry lung weight.

### **2) Individual particle analysis Scanning electron microscopy/ X-ray Spectrometry (SEM/XRS)**

Accurately weighed samples (in the range of 1 to 2 grams) of wet lung were freeze-dried to constant weight. Each specimen was then ashed for seven hours in a low temperature ashers. A suspension of the ash from each sample was made using 50 ml of a 0.05% solution of Aerosol OT in filtered, de-ionized water. The resulting suspension was sonicated in an ultrasonic bath for 15 minutes. The suspension was than made up to a final volume of 100 ml with filtered, de-ionized water to which 1 ml of glacial acetic was added and allowed to sit overnight. This removed most of the endogenous calcium and phosphorus containing particles. A sample blank was carried through the entire preparation process for each specimen. Aliquots of the suspensions for sample and blank were filtered onto 25 mm diameter, 0.1  $\mu\text{m}$  pore size polycarbonate filters for microanalysis.

Particles found on the filter preparation for each lung and blank were analyzed using a scanning electron microscope (Hitachi S-570) equipped with a Kevex 7000 energy dispersive x-ray analysis system and an Advanced Research Instruments Autosem image analysis system using the back scattered electron image. For each lung filter preparation, at least 1000 particles in a minimum of 20 randomly selected fields of view at a magnification of 1000X were analyzed. Particles found in the same number of randomly selected fields of view for the blanks were also analyzed and subtracted from the corresponding lung analysis.

The image analyzer uses a regular grid point spacing pattern with a preset point density (off point density) to locate particles. At each off point, the back scattered electron signal is compared to a threshold to determine whether the electron beam is on a feature of interest. For our analysis, this off point density was set at 0.1  $\mu\text{m}$ . Consequently, all particles 0.1  $\mu\text{m}$  and larger in any dimension will contain a grid point and be counted if the backscattered electron signal from the particle is above the threshold. Features smaller than 0.1  $\mu\text{m}$  can fall between the grid points and be missed. Once a particle is found, a narrower point density (on point density) is used to

determine various particle size parameters. Subsequently, each particle was analyzed for 31 elements using an X-ray spectrum acquired for 5 seconds. The particles were classified into mineral groups based upon the major elemental components and net fractional x-ray intensities (Stettler et al 1991; Green et al, 1990). In this paper only silica particles and aluminum silicate particles are reported. Final lung particle concentrations were calculated from the relationship between the weight of dry lung tissue ashed, the aliquot of the lung ash used to prepare the filter, the total filter area and area of the filter analyzed and the total number of particles analyzed for each filter.

## References

- Green FHY, Yoshida K *et al.* 1990. Characterization of airborne mineral dusts associated with farming activities in rural Alberta, Canada. *Int Arch Occup Environ Health* 62:423-430.
- Pooley FD. 1981. Tissue mineral identification. *Occupational Lung Disease: Research Methods and Approaches*. H. Weill and M. Turner-Warnick, eds. New York, Marcel Dekker.
- Stettler LE, Platek SF, Riley RD, Mastin JP, Simon SD. 1991. Lung particulate burdens of subjects from the Cincinnati, Ohio urban area. *Scanning Microsc* 5:85-92.